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Extended Summary
Drug and Pesticide Metabolism II: New Approaches in Metabolism and Toxicology of Agro- and Industrial Chemicals

The following extended summary is based on a paper presented at the above meeting organised by the SCI Physicochemical and Biophysical Panel of the Pesticides Group and the Drug Metabolism Group, and held on 18 December 1995 at St Mary's Hospital Medical School, Paddington, London. It is entirely the responsibility of the author and does not necessarily reflect the views of the Editorial Board of Pesticide Science.

Biochemical Markers of Genotoxicant Exposure, with Particular Reference to Pesticides

Peter B. Farmer

MRC Toxicology Unit, Hodgkin Building, University of Leicester, PO Box 138, Lancaster Road, Leicester, LE1 9HN, UK

The use of biochemical markers to monitor human exposure to genotoxic chemicals ('biomonitoring') is now well established.[1] To date, most emphasis has been placed upon monitoring chemicals from occupational sources and from the wider environment (e.g. from tobacco smoking and urban pollution). Relatively little attention has been paid to pesticides and other agrochemicals.

The currently accepted mechanistic pathway for genotoxic carcinogens involves the initial formation of a covalently bound product (adduct) with DNA, which may lead to a mutation. Considerable technical advances have recently been made in both physicochemical/biochemical approaches for determination of adducts and in molecular biological methods for mutation detection. The former methods give specific information on the chemical involved in the exposure, and yield the 'biologically effective doses' of these chemicals. In contrast, the detection of mutations is much less likely to identify the genotoxic chemical(s) involved, but nevertheless has the advantage that one is now looking at an adverse biological effect resulting from adduct formation, which may be more relevant than adduct measurements for risk estimation. (Measurement of later biological effects, such as chromosomal aberrations, may also be considered in an analogous way to mutation detection.)

The formation of an adduct in DNA involves the interaction of an electrophilic species derived from the carcinogen with a nucleophilic site in DNA (e.g. N or O in the nucleic acid bases or O in the internucleotidic phosphates).[1] Some genotoxic carcinogens are intrinsically electrophilic, but many require metabolism to generate such species. Reaction of these electrophiles is not confined to DNA, and formation of reaction products with protein, glutathione and water (hydrolysis) also occurs. In general, protein and peptide adducts have no toxicological significance, the exception being for adducts with some dihaloalkanes, which still possess electrophilic activity.[2]

The main analytical approaches for determination of DNA-carcinogen adducts are ^{32}P-postlabelling, gas chromatography–mass spectrometry (GS–MS), HPLC with fluorescence or electrochemical detection, and immunoassay. ^{32}P-postlabelling offers the most sensitive and probably the most general approach for monitoring genotoxic damage.[3] To carry this out, the DNA is digested enzymatically to 2′-deoxynucleoside 3′-

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monophosphates. Carcinogen-adducted nucleotides are partially purified from the normal nucleotides ('enrichment') and then phosphorylated enzymatically at the 5′-position using ^{32}P-ATP. The resultant 3′,5′-bisphosphates are chromatographed (TLC or HPLC) to resolve the adducts which are then quantified by their radioactivity content. The procedure is applicable to most genotoxic carcinogens and has been used to detect less than 1 adduct per 10^8 nucleotides. The application of ^{32}P-postlabelling to monitor pesticide–DNA interactions has not been extensive. A recent example was the detection of DNA adducts in non-healthy hops in heptachlor-contaminated fields.[4] A second example is from Bolognesi *et al.*,[5] who recently showed that 'Lannate' 25, a commercial methomyl-containing formulation, yielded DNA adducts in livers of treated mice, whereas pure methomyl yielded no adducts. The compound responsible for the adduct formation was not identified.

A further approach of particular value for monitoring DNA damage in humans exposed to genotoxic agents is the detection of urinary alkylated purines.[6] Many electrophilic (and especially low-molecular-weight) alkylating agents form adducts at the N-7 position of guanine and the N-3 position of adenine. These modified bases are removed from the DNA by enzymatic repair processes and excreted in urine. They may then be quantitated immunochemically or by GC–MS. A few volatile agrochemicals are known to be alkylating agents (e.g. methyl bromide, ethylene dibromide and dichlorvos), and there seems to be some potential for determining the extent of human exposure to compounds of this type from studies of urinary alkylated purines, (see also reference to protein adduct formation below). Background levels of adducts of some low-molecular-weight alkylating agents (e.g. methylating agents) with DNA exist, which may limit the sensitivity of adduct measurement to confirm exposure.[1] The source of these background levels of adducts is unknown, and may include both endogenous and exogenous exposure to alkylating species.

The main analytical approaches for determination of protein–carcinogen adducts are MS and (to a much lesser extent) immunoassay.[1] The proteins most commonly chosen for such measurements are haemoglobin and albumin, because of their ready availability and long lifetime. The sites of adduct formation are the nucleophilic amino acid side chains (e.g. SH, COOH, imidazole) and, in the case of haemoglobin, the valine *N*-terminal amino group. Modern mass spectrometric techniques, e.g. electrospray MS–MS, are capable of identifying the location of adducts within the protein chain.[7] Quantitation of protein adducts is generally carried out by GC–MS(–MS) following chemical degradation of the protein chain. A modified Edman degradation procedure has proved particularly suitable for detection of *N*-terminal haemoglobin adducts (e.g. from ethylene oxide,[8] acrylonitrile,[9] etc.). Alternatively, complete acidic hydrolysis of the protein chain results in generation of all the amino acids. From such a mixture, modified amino acids may be separated chromatographically and subjected to GC–MS quantitation. Such a procedure was used, for example, by Iwasaki *et al.*[10] to monitor workers exposed to methyl bromide. Mild acidic or alkaline hydrolysis of adducted proteins results in the cleavage of some cysteine and aspartic and glutamic acid adducts, liberating carcinogen-derived species which may be extracted and analysed by GC–MS. Thus Sabbioni and Neumann[11] have developed sensitive approaches for determination of haemoglobin adducts of aromatic amines derived from pesticides. The protein was hydrolysed in base, which released the aromatic amine from its cysteine adduct, followed by derivatisation for GC or GC–MS determination. A second example of this type of approach is represented by the recent elegant demonstration by Pastorelli *et al.*,[12] who showed that ethylene thiourea (ETU) (which is a main degradation product of ethylenebis(dithiocarbamate) pesticides) formed an adduct with haemoglobin. Acidic hydrolysis of this protein released ETU which was quantitated by stable isotope dilution GC–MS. The presence of this adduct was found in the haemoglobin of some workers exposed to mancozeb.

Although agrochemicals as a class do tend to be non-genotoxic, as demonstrated in studies for registration, the potential of DNA and protein adduct measuring techniques for detection of human exposure, particularly for workers, to those which are genotoxic is significant, and further use of these approaches seems likely in coming years. Humans are constantly exposed to a whole array of DNA-damaging agents (as witnessed by the presence of many 'background' adducts in human DNA), and the contribution of agrochemicals to this genetic burden remains to be fully established.

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